Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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Chapter 10: Topoisomerase II is a target of anti-cancer drug action.

Introduction

The DNA double helix is naturally twisted. Normally, it has one full twist for every 10 base-pairs. But what happens to the twists when the DNA strands are pulled apart during replication or transcription (Figure 10.1)? It is like trying to pull apart a long 2-strand twisted rope. The twists bunch up and it becomes harder and harder to pull the 2 strands of the rope apart. It was at first hard to imagine how a cell could solve that problem. These topological problems are solved by topoisomerases of type I. These enzymes produce transient single-strand breaks, and allow the strands to swivel about each other towards their stable configuration of twists.

Another problem happens when the DNA has replicated and the chromosomes begin to condense on their way to mitosis. The mother and daughter strands become entangled in a manner that pulling the long DNA stands apart becomes like trying to pull 2 interlocked rings apart. It can't be done without cutting one of the rings. Topoisomerases of type II solve that problem by transiently cutting both strands and allowing an intact double helix to pass through the gap.

Interest in topoisomerases blossomed from the discovery that some important anticancer drugs work by blocking one or another of those two types of topoisomerases.

This chapter is about drugs that block type II topoisomerase, which were the first drug-topoisomerase actions to be discovered. The following chapter (Chapter 11) will be about drugs that bock type I topoisomerases.

Discovery

It is my pleasure to tell how the early discoveries of drug actions on topoisomerases were made, because my colleagues and I had a hand in making them.

The early discoveries of the topoisomerase enzymes, however, were made before the drug actions on them were known. Topoisomerase type I enzymes were first to be discovered. They were discovered in bacteria and viruses and were initially called "DNA nicking-closing enzymes" or “DNA swivelases” (Champoux, 1978b; Champoux and Dulbecco, 1972; Radding, 1978). As explained by Champoux, the enzymes "introduce a transient single-strand break in duplex DNA and thereby provide a swivel for helix unwinding (DNA swivelase)" (Champoux, 1978b). Those names were later replaced by "topoisomerase" to indicate that the enzymes change the DNA topology (a change in topology occurs when the object has to be cut to make the change). Topoisomerase I, however, is the subject of the next chapter (Chapter 11), because drug actions were first discovered on topoisomerase II, which is the subject of the current chapter .

Type II topoisomerases cleave both strands of the DNA so as to form a double-strand break through which another double-stranded DNA can pass before the enzyme reseals the break (Liu et al., 1980; Miller et al., 1981). This amazing ability is important during and after DNA replication, because the new chromosomal DNA would otherwise remain entangled in loops analogous to the interlocking circles in the symbol of the Olympics (Figure 10.1), the interlocking would hinder the proper separation of chromosomes during mitosis. How topoisomerase II accomplishes that trick will be explained later in this chapter.

Figure 10.1. The interlocking ring symbol of the Olympics (jeux olympiques). After being replicated, DNA tends to be catenated like these interlocking rings. Topoisomerase II undoes those catenated tangles in newly replicated chromosomes.

First clues of anticancer drugs acting on topoisomerases.

As already mentioned in the previous chapter (Chapter 9), the first evidence for drug actions on a topoisomerase came from our DNA filter elution experiments. It turned out that our drug findings were of actions on topoisomerase II, an enzyme that had not yet been discovered.

Here is how the initial discovery of drugs actions on topoisomerase came about: In 1978, a young physician, Warren E. Ross, having completed his first year as a Clinical Associate in the National Cancer Institute, joined my laboratory to gain some research experience. At that time, we were studying DNA damage and repair produced by various anticancer drugs in cells. We had developed a new technique using filters that allowed us to measure DNA stand breaks and DNA crosslinks, both between the paired strands, and between DNA and proteins (Kohn and Ewig, 1979). The story of that technique was told in the previous chapter (Chapter 9).

Warren wanted to apply that methodology to doxorubicin, a promising drug that interested him in his Clinical Associate year. Doxorubicin had been reported to break DNA strands in studies that used the previous less precise and less sensitive ultracentrifugation method. We fully expected that using our new filter-based technique, we would easily confirm the production of DNA breaks by doxorubicin in mammalian cells, as had invariably been the case with several other DNA-breaking agents that we had tested (Erickson et al., 1977; Fornace et al., 1976). However, Warren's repeated attempts to confirm doxorubicin-induced DNA breaks using our filter method failed to show any sign of DNA breakage whatsoever (arrow in the *left* panel of Figure 10.2).

His experiment however suggested that doxorubicin produced DNA-protein crosslinks: the lower two curves in the *left* panel of Figure 10.2, showed that using x-rays to produce strand breaks yielded less than the expected rate of elution (see legend to Figure 10.2) . We thought that doxorubicin failed to show any DNA strand breaks because the drug might have produced an excess of DNA-protein crosslinks, which could have hidden the strand breaks -- because the bound proteins might have caused all of the DNA fragments to stick to the filter.

That idea seemed to be confirmed, because digesting the lysed cells with a proteinase before alkaline elution, produced an increased elution rate that seemed indicative of the expected strand breaks (*right* panel of Figure 10.2). Moreover, when Warren applied our protocol for protein-digestion (see Chapter 9), the results were astounding. We had never seen anything like it: doxorubicin then produced a beautiful pattern of dose-dependent strand breakage (Figure 10.3). But protein digestion was needed to reveal those breaks – because the DNA fragments were completely hidden by being linked proteins that bound to the filter.

In order to hide the strand breaks so completely, however, we thought a large excess of DNA-protein crosslinks relative to strand breaks would be needed. We were able to check on that, because we had recently worked out how to quantify both strand breaks and DNA-protein crosslinks (Chapter 9) (Kohn and Ewig, 1979).

The results of those quantifications presented a big surprise and a puzzle. They showed that there was NO excess of DNA-protein crosslinks over strand breaks. In fact, repeated measurements with doxorubicin, as well as some other DNA intercalators (such as ellipticine) consistently showed that the number of the two types of DNA lesions were equal, within experimental error!

That seemed amazing and strongly indicated that there was some connection between the strand breaks and the DNA-protein crosslinks. They must have been causally connected in some way.

The next notion that dawned was that maybe the DNA-linked protein was actually an enzyme the produced the strand break and that the drug caused the enzyme to remain linked to one end of the break it produced. Then every DNA strand segment would have a protein linked to it and the number breaks and DNA-protein crosslinks would be equal, as observed in our experiments (Ross et al., 1979).

It is not often that one experiences the delight of imagining something important that perhaps no one had thought of before and having it come to fruition.

But to verify that idea required some calculation. Three models could be considered for the distribution of the strand breaks and the DNA-protein crosslinks) (Ross et al., 1979). The models are described in Figure 10.4. Model I assumed a random distribution of both strand breaks and DNA-protein crosslinks; this model failed, because and equal frequency of the two DNA lesions would have left some DNA strands without protein links, contrary to our evidence. Model III assumed one protein bound to every strand segment, but anywhere along the segment; this seemed an unlikely circumstance, because it was difficult see what could have brought about such an arrangement. Model II was plausible if the linked protein was and enzyme that produced the breaks and if a drug, such as doxorubicin, remained attached to one end of the break it produced.

To recapitulate, the equal frequency we observed of doxorubicin-induced strand breaks and DNA-protein crosslinks was at first puzzling, because if the two types of DNA lesions were randomly distributed along the DNA, some broken strands would by chance have been free of protein and therefore should have passed though the filter, contrary to our observations. We then reasoned that the breaks could have been completely hidden, as observed, if there were just one protein linked to each broken strand. That, at first seemed unlikely, but we soon realized that it could be the case if each protein molecule were bound consistently to one end of each break site (Figure 10.4, Model II). Algebraic analysis of our data was consistent with that possibility (Ross et al., 1979; Ross et al., 1978).

As already said, if a protein were linked consistently to one end of each strand break, then perhaps the protein was an enzyme that produced the break. An enzyme with that property was already known in bacteria: it was called a nicking-closing enzyme, because it produced the breaks transiently and was able to reseal them (Champoux and Dulbecco, 1972).

We therefore proposed that a type of nicking-closing enzyme existed in mammalian cells and that doxorubicin (as well as other DNA intercalating agents that we observed to produce similar results (equal numbers of DNA strand breaks and DNA-protein crosslinks) caused the enzyme to become blocked in an intermediate state where the break had been produced but had not yet resealed. Therefore in 1979 we "proposed that intercalation-induced distortion of the DNA helix leads to strand scission by a nuclease which becomes bound to one terminus of the break so as to form a DNA-protein crosslink" (Ross et al., 1979). Nicking-closing enzymes (also called "swivelases" or "DNA unwinding enzymes"), were soon found in mammalian cells (Champoux, 1978a) and were later dubbed “topoisomerases.”

These studies gave the first clue that drugs, such as doxorubicin, trap a topoisomerase in a state where the DNA strands are cleaved while the enzyme remains bound to the ends of the broken strands.

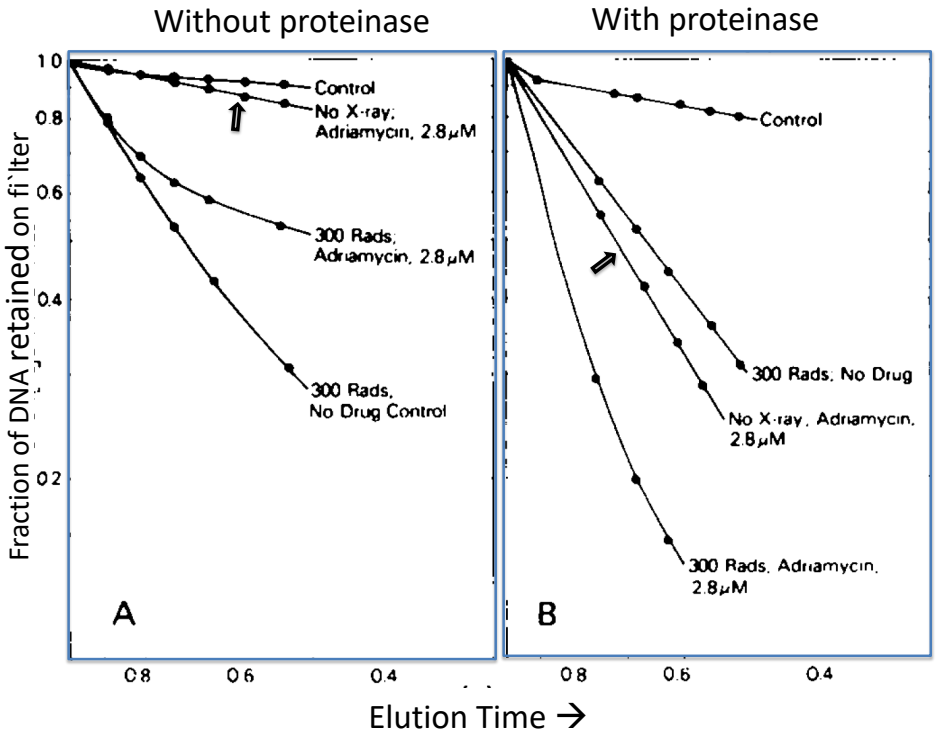

Figure 10.2. This experiment by Warren E. Ross in 1977 in my laboratory showed, surprisingly, that doxorubicin (Adriamycin) at first showed no increase in DNA alkaline elution rate, thus no indication of any strand breaks (arrow in *left* panel). However, the elution rate after subjecting the cells to 300 rad just before lysis and elution was reduced in the doxorubicin-treated cells, which suggested the presence of DNA-protein crosslinks (lower two curved in the *left* panel). When the assays included digestion of the lysed cells with proteinase, however, doxorubicin showed the increase elution expected for the presence of DNA stand breaks (arrow in the *right* panet). All together, these results suggested that doxorubicin produced both strand breaks and DNA-protein crosslinks (Ross et al., 1978).

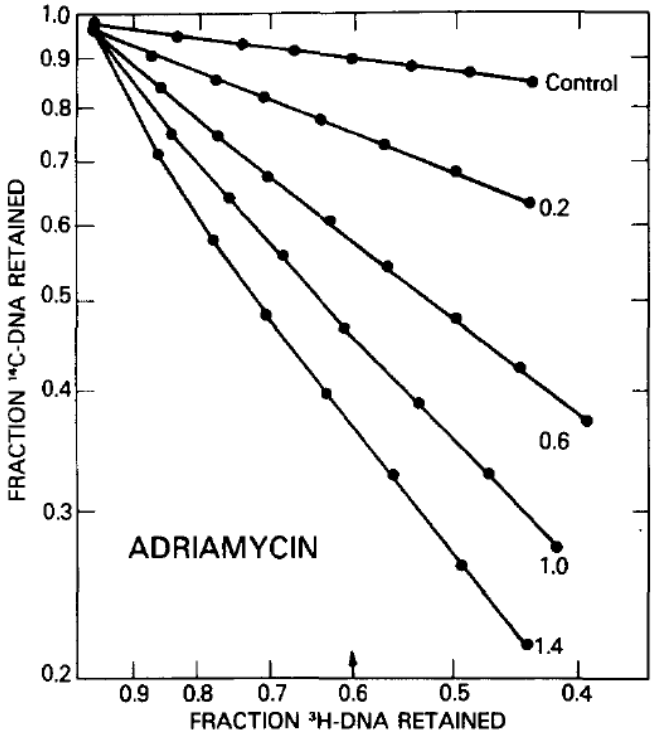

Figure 10.3. Demonstration of protein-linked DNA strand breaks in cells treated with doxorubicin (adriamycin). In this experiment, doxorubicin-treated cells lysed on the filter were subjected to a protein-digesting enzyme before pumping an alkaline solution through the filter. DNA strand breaks in proportion to the doxorubicin dose are revealed. (Without the protein-digestion step, no DNA strand breaks could be seen (curve similar to that labeled “control”) (Ross et al., 1979) (Ross et al., 1979).

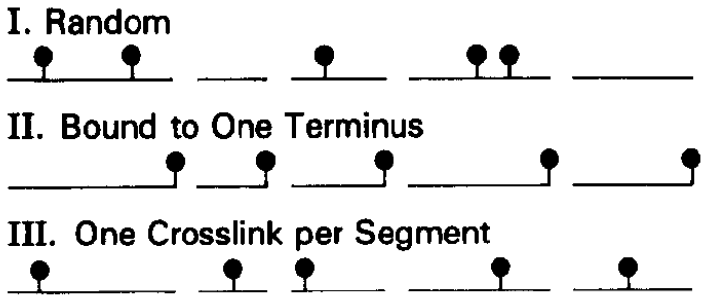

Figure 10.4. Three models could be considered to account for our observation that doxorubicin (as well as some other DNA intercalators, such as ellipticine) produced equal numbers of strand breaks and DNA-protein crosslinks. The lines in the diagrams represent DNA strands with interruptions at break sites. The black circles represent protein molecules bound to the DNA strands. Model I was for proteins bound at random places on the DNA; note that by chance some broken DNA pieces have no protein attached. Model III for one and only one protein randomly placed on each DNA segment was unlikely, because how would the linked protein know where the breaks were located? Model II was for a protein bound consistently to one end of each break. Quantitative examination of the data was consistent with Model II. The conclusion that there was a protein bound consistently to one end of each break (Model II) suggested that the DNA-bound protein molecules in fact produced the breaks (Ross et al., 1979).

The next step would be to demonstrate the effect of the drugs on the purified topoisomerase enzyme or in solutions extracted from cells containing the enzyme. Janek Filipski, a Polish visiting scientist in our lab experienced considerable frustration in this work. He succeeded in showing that cell extracts contained an enzyme that produced the expected drug effects – DNA stand breaks with associated DNA-protein crosslinks. However, when he tested purified topoisomerase, the drugs had no effect (Filipski et al., 1983a, b). Soon after he published his work, the problem was revealed: there were two kinds of topoisomerases, and he was testing the wrong one. Only topoisomerase I was known at the time of his experiments. The enzyme the drugs he was testing acted on was only topoisomerase II, which was being discovered, unknown to us, during the latter part of his studies.

In 1980, Leroy Liu, working with Bruce Alberts at the University of California in San Francisco, had isolated an enzyme that came to be known as topoisomerase II (Liu et al., 1980). About 2 years later, after Leroy Liu had moved to Johns Hopkins University in Baltimore, I visited his laboratory and we discussed the possibility that the drug effects that we could not attribute to actions on topoisomerase I were actually due to his topoisomerase II. After preliminary experiments to get the drug treatment conditions right, Leroy Liu and his colleagues, as well as John Minford, Yves Pommier and Len Zwelling in my laboratory, soon confirmed that indeed

doxorubicin trapped topoisomerase II bound to one end of a DNA break, an intermediate state in the enzyme's breakage/resealing action (Minford et al., 1986; Nelson et al., 1984; Tewey et al., 1984a; Tewey et al., 1984b). In addition to doxorubicin, we found that some other DNA intercalating drugs, such as amsacrine (m-AMSA) and ellipticine, also trapped topoisomerase II DNA-cleavage complexes in a fashion similar to doxorubicin (Pommier et al., 1985).

The nature of the protein-associated DNA strand breaks that we attributed to trapping to topoisomease II was further revealed by studies of the action of amsacrine (m-AMSA) by Len Zwelling in my laboratory (Zwelling et al., 1981). Len added m-AMSA to cultures of mouse leukemia cells and measured the production of protein-linked DNA strand breaks using our filter methods (Kohn, 1996) (Figure 10.4). If m-AMSA produced DNA breaks like an ordinary DNA damaging agent, the breaks would continue to accumulate while active agent was present. He found, however, that the breaks produced by m-AMSA soon leveled off, and then remained at a constant level as long as the drug was present. When the drug was removed, the breaks rapidly vanished. We concluded that, in the presence of m-AMSA, there was a rapid equilibrium between formation and reversal of breaks. The simplest explanation was that the drug trapped an intermediate state of an enzyme that continually opened and closed DNA breaks.

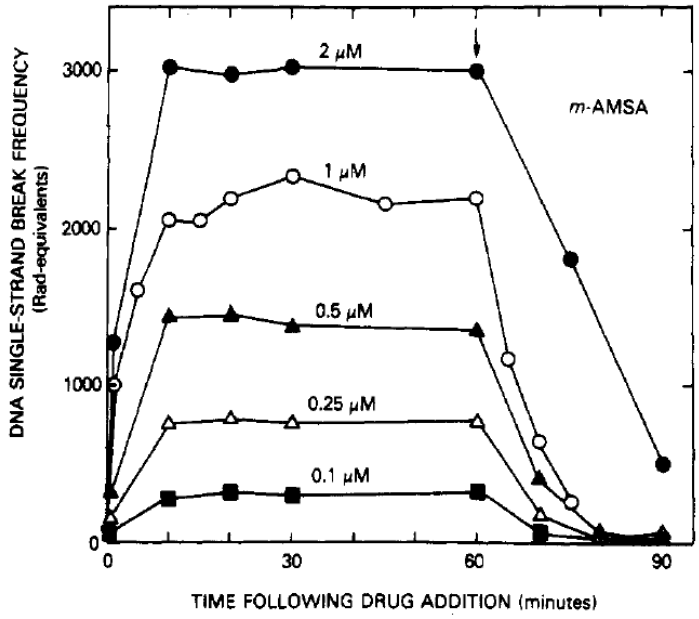

Figure 10.4 m-AMSA (amsacrine) causes DNA strand breaks to appear and reseal rapidly, consistent with an effect on a topoisomerase. The number of strand breaks increased and soon reached a plateau that was higher when the drug concentration was higher. After 60 minutes, when the drug was removed (arrow), the strand breaks soon vanished. This result showed that there was a rapid equilibrium between formation and reversal of the strand breaks, and the number of strand breaks at equilibrium increased with drug concentration (Zwelling et al., 1981). An

ordinary DNA damaging agent would have continued to increase the number of strand breaks, in contrast to the flat equilibria seen here.

Later it turned out that another drug, camptothecin, trapped topoisomerase I in a rapidly reversible where a only one of the strands of the DNA double helix was cleaved. Topoisomerase I, like topoisomerase II undid excessive DNA twists, but did by producing DNA singe-strand breaks, as opposed to the double-strand breaks produced topoisomerase II. The camptothecin story is related in the next chapter. (Chapter 11).

How doxorubicin and other intercalator-type drugs trap DNA-topoisomerase II complexes.

In 1989, when purified topoisomerase II and DNA sequencing gels had become available, we wondered whether the drugs had preferences for the DNA sequences where they incited the enzyme to cleave the DNA. We found DNA cleavage did occur at particular sites (Figure 10.5). In order to determine whether the enzyme preferred to cleave in particular DNA sequence neighborhoods, we examined a large number of topoisomerase II DNA cleavage sites trapped by various intercalator-type drugs (Capranico et al., 1990a; Capranico et al., 1990b) (Pommier et al., 1991). Figure 10.5 shows one of our first DNA sequencing gels that indicated exactly where in the DNA sequence the drug-induced cleavage sites were located. When we began that investigation, however, we did not suspect that it was to give a clue to the structure of the trapped DNA-topoisomerase complexes.

Our first notable observation was that doxorubicin breaks occurred preferentially where there was an A (adenine) adjacent to the cleavage site on the side toward the 5' end of the DNA strand. For amsacrine (m-AMSA) there was also a preference for a particular base at the cleavage site, but in this case, the preference was for an A on the side towards the 3' end of the broken DNA strand. For etoposide and teniposide (VP-16 and VM-26), again there was a preference for a particular base at the cleavage site, but the preference in this case was for a C on the 5' side (Figure 10.6).

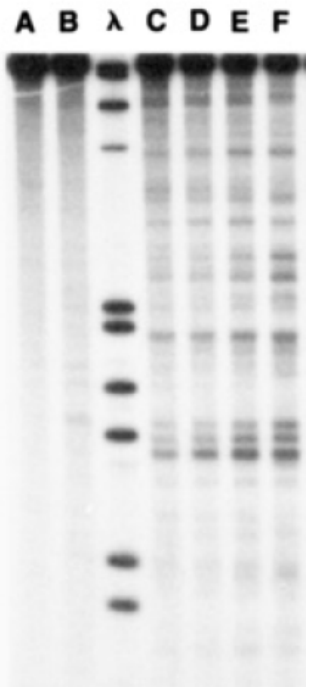

Figure 10.5. One of our first electrophoretic DNA sequencing gels showing cleavage of DNA at specific sites induced by mammalian topoisomerase II in the presence of doxorubicin (Capranico et al., 1990b). **A**, DNA alone. **B**, DNA plus topoisomerase II; these 2 lanes show that neither DNA alone nor topoisomerase alone nor DNA with only drug, caused breaks. **C-F**, DNA plus topoisomerase II plus increasing concentrations of doxorubicin; the bands show where in the DNA sequence cleavage occurred in the presence of topoisomerase II plus doxorubicin. As the concentration of doxorubicin was increased, the bands became darker, indicating increased frequency of breaks at those sites. (The lane labeled λ shows marker bands for determination of the exact positions of the cleavage sites in the DNA sequence.)

The preference for a base on one side or the other of the break site, and its dependence on the identity of the drug, suggested that the drug molecule stacks against one side of the other of the break site the way DNA intercalators stack against the base-pairs (Pommier et al., 1991; Pommier et al., 2000). We guessed (correctly) that the drug stacked against a particular base-pair at the cleavage site, as shown in (Figure 10.6).

The insertion of the intercalated drug prevents the topoisomerase II from closing the DNA break. The drug thus traps the DNA-topoisomerase complex in a state where the DNA is cleaved and cannot reseal. Since the bindings are reversible, the drug eventually dissociates and allows the break to reseal. The cell however does not rely on the spontaneous dissociation of the drug, because it takes some time, during which an encounter with a transcription or replication fork could have lethal consequences, as will be explained later in this chapter. The cell therefore has repair machinery to clean up (albeit slowly) the trapped complexes.

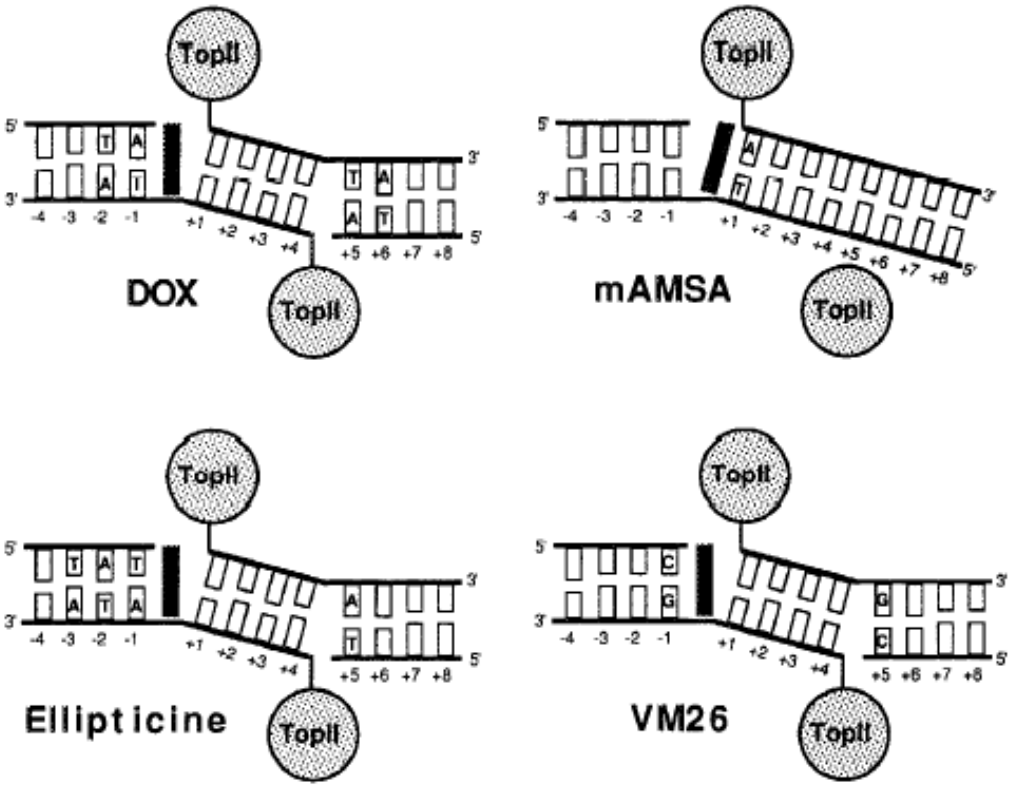

Figure 10.6. Preferred positions of the drugs (solid rectangles) at the drug-topoisomerase II cleavage sites. We inferred these configurations from our observed site preference observations (such as shown in Figure 10.5 (Pommier et al., 1991)). This model was later confirmed by x-ray crystallography (Wu et al., 2013). The DNA base preferences for the immediate neighbors at the break site were, as indicated in the figure: for doxorubicin (DOX), A on the 5' side of the break; for amsacrine (m-AMSA), A on the 3' side; for ellipticine, T on the 5' side; for teniposide (VM26) and etoposide (VP16), C on the 3' side. Topoisomerase II consists of 2 identical molecules bound together (Figure 10.7, but here shown separately), one cleaves one DNA strand, and the other cleaves the other strand. The 2 cleavage sites are always separated by 4 base-pairs, and the base preferences are similar at the 2 sites.

How type 2 topoisomerases undo entangled DNA helices.

The problem of separating interlocked newly replicated DNA loops (Figure 10.1) at first seemed almost insurmountable, but topoisomerase II manages to do it! It is like a conjuring trick that passes one rope through the middle of another. How one DNA double strand could be made to pass through another, while keeping hold of the strand ends, was at first hard to imagine. But, as so often is the case, evolution discovered a solution, which turned to be quite simple.

It was discovered that it happens through the cooperation of two identical topoisomerase II molecules (Figure 10.7): the topoisomerase molecules first cut one DNA double-helix (green), then allow the other (red) to pass through the gap and out the other side; then the molecules quickly and perfectly make the green DNA whole again. It happens quickly and perfectly, as if by magic. The key is that two topoisomerase molecules cooperate so that cut DNA ends are always bound to the topoisomerases and never free to drift away. And that the topoisomerase II pair of molecules have two places where they can bind each other alternately to let the passing double helix come in from one direction and out the other way (Figure 10.7).

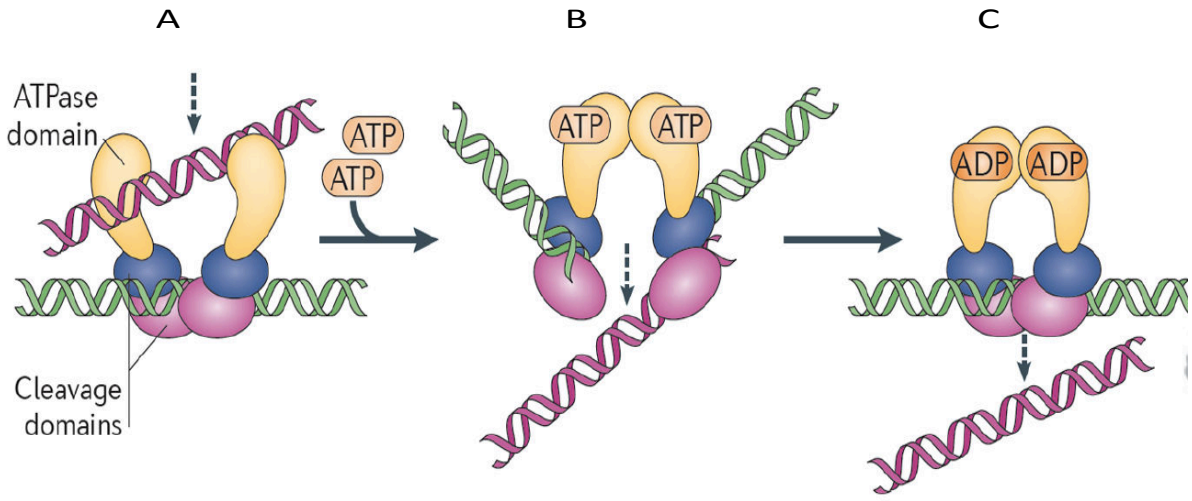

Figure 10.7. How topoisomerase II passes one DNA double helix (red) through another (green) (Vos et al., 2011). Two identical topoisomerase molecules cooperate to accomplish this magic. The ATP/ADP units provide the energy that drives the machine. (*From Nature Reviews Molec Cell Biol 2011 -- permission needed.)*

Doxorubicin and other DNA intercalation-type drugs bind to the intermediate state (B, in Figure 10.7), where the DNA is broken; the bound drug prevents the break from being resealed. Figure 10.8. shows the structure of this intermediate state as revealed by x-ray crystallography; we were very happy to see our conjectured model (Figure 10.6) confirmed by x-ray crystallography (Figure 10.8).

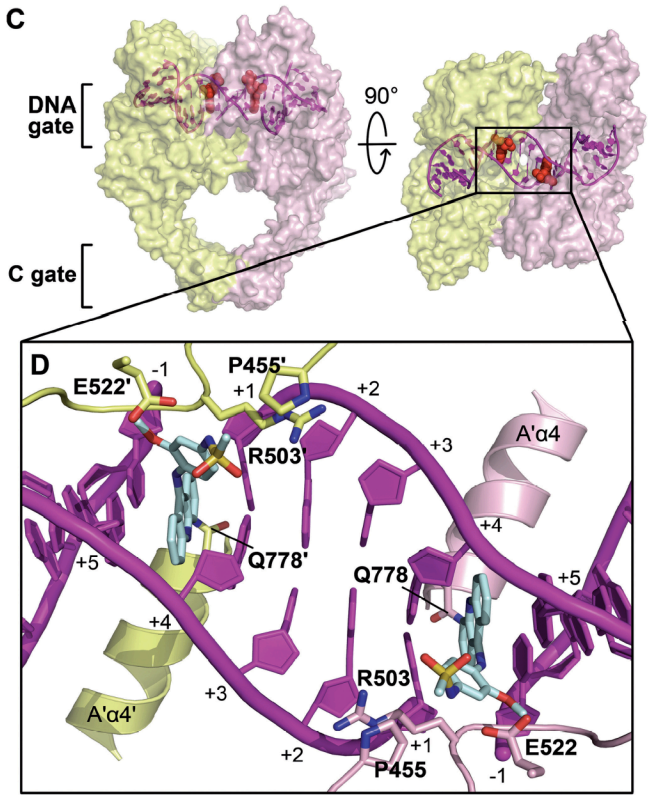

Figure 10.8. Structure of DNA-topoisomerase II trapped by amsacrine (m-AMSA) in a state where both DNA strands are cleaved (Wu et al., 2013). The structure is based on x-ray crystallography. The upper part of the figure shows the topoisomerase II homodimer (yellow) and the bound DNA (red). Below is a detailed view of the cleaved DNA with intercalated amsacine. The DNA (red) is shown with the base-pairs edge-on, connected to the DNA backbone via the pentagonal deoxyribose units. Two amsacrine molecules (blue) are DNA-bound at the two break sites, which are separated by 4 base-pairs. In the absence of drug, those 4 base-pairs come apart and the DNA double-strand break opens and allow another DNA helix to pass through. The complementarity of those 4 base-pairs then helps the two parts of the broken strand to find each other and restore the original unbroken DNA. Two alpha-helical parts of the topoisomerase II protein that interact with the DNA and/or amsacrine at the break sites are shown in yellow (Wu et al., 2013). *(WuC'ChanN'13nar-Top2-amsa-cryst.pdf -- Nucleic acids research 2013, permission needed.)*

How drugs that poison topoisomerases kill cancer cells.

You might think that the toxic effects of a drug that poisons an enzyme would be overcome if the cell increased the amount of the enzyme, so that some enzyme activity would still be retained even in the presence to the drug. According to that viewpoint, cells would become resistant to the drug if the amount of the drug's

target enzyme were increased, which is often the case of other enzymes. However, for topoisomerases the opposite is true. Cells become drug-resistant if they *reduce* the amount of topoisomerase they make, because it is the drug-topoisomerase combination that is toxic to the cell (Nitiss, 2009) (Pommier, 2013). This situation is sometimes called "synthetic lethality", because increased synthesis of the drug's target makes cells more sensitive to the drug, *i.e.*, more easily killed by the drug.

But why would a drug-topoisomerase complex, sitting quietly on the DNA cause trouble? The trouble arises when a DNA replication or transcription machine comes along and encounters one of those complexes. The encounter creates an abnormal DNA structure, such as a double-strand end, which is hard to repair, and such lesions in the DNA can ultimately kill the cell (Hsiang et al., 1989).

How the cell defends against drugs that poison topoisomerases.

The best defense of course is prevention. The cell does that by means of enzymes that remove the trapped topoisomerase from the DNA before anything bad happens. A second mechanism, DNA nucleotide excision repair, comes into play at sites where a DNA or RNA polymerase process has already collided with a drug-topoisomerase complex on the DNA. A third defense is initiated by signals to the cell cycle control systems to delay replication and mitosis, so as to give more time for repair to take place before disastrous consequences occur. If there are too many trapped complexes to handle, however, the cell may give up and undergo programmed cell death (apoptosis).

The first countermeasure mentioned above, prevention, is fairly well understood. It is accomplished by enzymes called tyrosine-DNA-phosphodiesterases (TDP1 and TDP2). Phosphodiester bonds normally link between nucleotide units in the DNA sequence (Figure 10.9). When a DNA-topoisomerase complex has cleaved a DNA strand, a phosphodiester bond, instead of restoring an intact strand, links one end of the cleaved DNA to a tyrosine amino acid of the topoisomerase. TDP1 and TDP2 juggle the phosphodiester bonds to make the topoisomerase protein come off (at which point the drug also comes off) and allows the DNA break to reseal. When the DNA strand break cannot close because of an intercalated drug, TDP1 or TDP2 breaks the bond between the DNA end the topoisomerase's tyrosine. The importance of this action was shown in a report that TDP2 helps cells survive topoisomerase II trapping by the Top2 blocker, etoposide (Kont et al., 2016).

Actually, the process is a bit more complicated. Before the TDP1 or TDP2 can cleave the tyrosine bond to the DNA, a large part of the topoisomerase protein has to be digested away. This is done by an important (and amazing) machine in the cell, called a proteasome.

Other types of DNA damage, such as produced by alkylating agents can also trap topoisomerases (Schellenberg et al., 2016), but that is generally a minor action relative to other effects of those agents.

The third defense: signaling to the cell cycle control system to delay replication and the initiation of apoptosis is the subject of Chapter .... Efforts aimed to unravel the complexities of how these DNA lesions, induced by topoisomerase-trapping drugs signal to the DNA repair and cell cycle control systems to initiate further survival efforts by the cell (Cristini et al., 2016) (Sakasai and Iwabuchi, 2016).

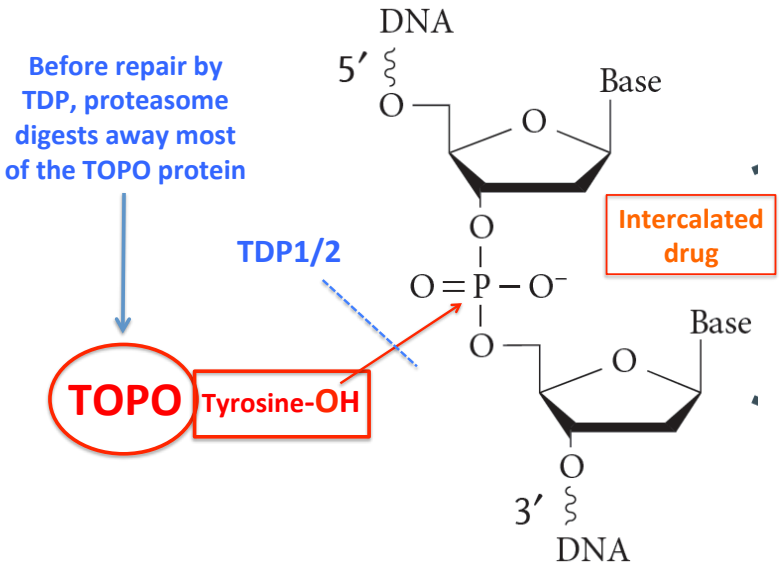

Figure 10.9. Formation of a trapped topoisomerase-DNA complex and its repair by proteasome and TDP. It is not as complicated as it looks. Here are the steps:
(1) The topoisomerase's tyrosine oxygen atom attacks the phosphorus atom (P) that joins 2 nucleotide units in a DNA strand (red arrow).
(2) At the same time that the tyrosine oxygen binds to the P, an oxygen atom in the DNA dissociates from the P, thereby producing a break in the DNA strand. The oxygen atom that dissociates from the P is either the one connected to the 5' part of the DNA or the one connected to the 3' part of the DNA, depending on the type of topoisomerase, but that is a minor point here.
(3) An intercalator-type drug binds to a base-pair adjacent to the strand break and prevent the resealing of the break, thereby trapping the topoisomerase. Some drugs bind to the base towards the 5' part of the DNA strand, and some towards the 3' part of the DNA strand, as shown in Figure 10.6.
(4) In the repair of the trapped complex, a proteasome first digests away most of the topoisomerase protein.
(5) Finally, TDP1 or TDP2 (depending on the type of topoisomerase) breaks the bond between the tyrosine oxygen atom and the DNA's P atom, while reforming the

bond between the P and the previously dissociated DNA oxygen atom. In the end, normal DNA structure has been perfectly restored.

The Etoposide Story

So far, all the Top2 blocking drugs mentioned had the ability to intercalate in DNA, which aided their discovery. But there is a different group of Top2 blockers. Here is the story.

It starts with Hartmann Stahelin and coworkers at Sandoz, who were manipulating the chemistry of podophyllotoxin, which was known to prevent cells from passing through metaphase of mitosis (Keller-Juslen et al., 1971). The drug was obtained by extracting it from the roots of a poisonous plant: the American mandrake or Mayapple (Figure 10.10). Podophyllotoxin had anticancer activity in mice but was found to be too toxic for use in patients. Therefore, the chemists at Sandoz made chemical modifications of the compound in search of a less toxic drug. They made almost 50 variations of the chemical structure of podophyllotoxin, several of which increased the survival of mice with leukemia L1210.

There was a big surprise, however, when a modest structural change in the podophylotoxin structure completely changed what the drug did in the cell: the toxicity to cell was retained, but the mechanism responsible was entirely different. Moreover, the altered drugs were much more effective against cancer.

The change was merely to remove a methyl group and switching the steric configuration of one of the bonds (Figure 10.11). This modest change eliminated (or greatly reduced) the ability of the drug to inhibit in metaphase of mitosis. Instead, the cells were prevented from even starting the process toward mitosis. This was reported by Stahelin in 1970, who surmised correctly that the demethylepipodophyllotoxins (the chemical name of the new compounds) killed cells by an entirely new mechanism (Stahelin, 1970). The new compounds were later discovered to block topoisomerase II.

We became accustomed to that unwieldy chemical name and were relieved to let it fade in memory when it was superseded by new names for the drugs: etoposide and teniposide. (You might suppose that the name “etoposide” referred to its action on topoisomerase, but it seems that name was applied before its action on topoisomerase was known!) Thinking back on this story, the switch in biological target of action produced by simple changes in chemical structure was remarkable and instructive. It challenged the presumption that the drugs with similar chemical structure would necessarily act on the same target.

Figure 10.10. The American mandrake or mayapple, a poisonous plant, whose roots were the source podophyllotoxin, an inhibitor of mitosis. Chemical modifications of the compound yielded the topoisomerase II blockers and anticancer drugs, etoposide and teniposide. (*Photograph from Wikipedia.*)

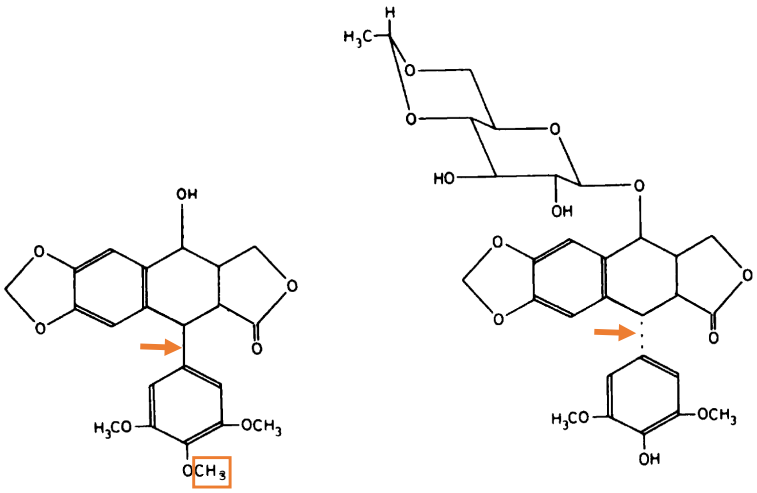

Figure 10.11. Chemical structures of podophyllotoxin and etoposide (VP16). The chemical changes need in order to switch the mode of action are (1) removal of the methyl (CH3) group (red square); and (2) change of the configuration of one of the bonds (red arrow). Teniposide (VM26) is a minor chemical modification of etoposide. (The chain in the upper part of the structure on the right is not essential to the change in drug action.)

Thus, the demethylepipodophyllotoxins surprised the researchers, because, although this modest chemical modification of podophyllotoxin increased the ability to extend the survival of mice with cancer, the new compounds did so by an entirely new action. Instead of blocking cells in the middle of mitosis, they instead blocked the ability of cells to begin condensing chromosomes as prelude to entry into mitosis (Grieder et al., 1974). Because of this drastic change in how the new compounds worked, they were given the tentative drug names, VP16 (later, etoposide) and VM26 (later, teniposide).

It was natural to suppose that cells were stopped from starting mitosis by inhibiting DNA synthesis. But the problem with that supposition was that the inhibition of entry into mitosis occurred sooner and at lower drug dose than the inhibition of DNA synthesis (Grieder et al., 1974). Therefore, something other than DNA synthesis inhibition had to be what caused the inhibited cell division. It was a puzzle.

Then, in 1976, Susan Horwitz (Figure 10.2) reported that etoposide produced DNA strand breaks that gradually disappeared, presumable by being repaired. But the cause and significance of that finding remained a mystery.

Eventually, etoposide and teniposide were shown to block topoisomerase II with a unique base-pair preference at the cleavage site (Figure 10.6) (Pommier et al., 1991). The mechanism seemed to involve an initial interaction between drug and enzyme, rather than between drug and DNA (Burden et al., 1996). Therefore, these topoisomerase II blocking drugs were inferred to act in a manner distinct from other drugs that have topoisomerase II as their target .

Etoposide became one of the most important anticancer drug and was often used in combination with cisplatin or cyclophosphamide; it was found to be particularly effective against small cell lung cancer and testicular cancer (Belani et al., 1994) (Meresse et al., 2004).

The TDP story: cutting off the fuzz at topoisomerase-DNA break sites.

In the presence of topoisomerase-blocking drugs, the normally transient DNA strand breaks cannot easily reverse and may produce a dead-end product from which the cell may not recover. The trouble is that the topoisomerase protein remains persistently bound to the DNA, where its presence blocks repair machinery from coming to the rescue. The topoisomerase cannot dislodge from the DNA in the normal fashion, because the drug, bound to the same site, prevents it from doing so.

The blocked topoisomerase becomes troublesome protein material. Protein-digesting machinery then comes into play and cuts away much of the bound topoisomerase molecule. However, the machinery leaves behind a DNA-bound protein fragment that it cannot access.

The remaining fragment of topoisomerase protein is finally cut away by a pair of enzymes, TDP1 and TDP2 (tyrosyl-DNA-phosphodiesterase 1 and 2.

The enzyme that came to be known as TDP1 and TDP2 was first discovered in 1996 in yeast by Howard Nash and his coworkers at NIH (Yang et al., 1996) (Pouliot et al., 1999). The process, as conceived by Howard Nash and his colleagues is diagrammed in Figure 10.12, and the process itself is explained in the Figure's legend.

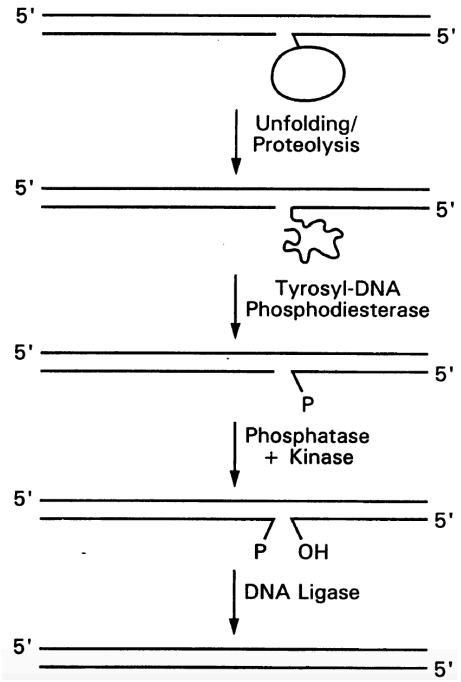

Figure 10.12. The process, as correctly surmised by Howard Nash in 1996, by which tyrosine-DNA-phosphodiesterase (later called TDP1 and TDP2) removes a drug-trapped DNA-topoisomerase fragment, so as to allow the DNA strand break to become repaired (Yang et al., 1996). Shown at the top is a partially digested topoisomerase fragment firmly bound to an end of a DNA strand break. (The strand break would originally have been produced by the topoisomerase in its normal function, but the enzyme would have become trapped by drug in a manner such that the enzyme could not spontaneously reverse and dislodge from the DNA.) Protein-digesting enzymes remove much of the topoisomerase protein, but leave behind a DNA-bound fragment that the protease cannot reach. TDP1 and/or TDP2 come in to finish the job. (Not quite correct in the last step is how the phosphates (P) are managed so as to allow the strand break to be repaired; the process is somewhat complicated and required considerable work to elucidate.)

So, what relevance would the TDP enzymes have for cancer therapy? On further study of the enzyme in yeast, Nash and his coworkers already in 1999 suspected that inhibition of TDP might increase the effectiveness of topoisomerase-inhibiting

anti-cancer drugs, because TDP would then not be available to cut away from the DNA break the potentially lethal protein fragment; persistence of the protein link to the DNA could kill the cell -- which would be good if it were a cancer cell that was killed (Pouliot et al., 1999). Therefore, much work was begun to discover TDP drugs that could be tried in cancer therapy together with topoisomerase inhibitors (Pommier et al., 2014).

TDP1 was found to process trapped topoisomerase I, and TDP2 was found to process trapped topoisomerase II (Pommier et al., 2014). The cell, therefore, was normally able to repair both types of topoisomerases trapped by drugs targeted to each of them. Hence, there were therapeutic possibilities for combining a TDP1 or TDP2 inhibitory drug with a drug targeted against the respective topoisomerase.

However, as usual, there was a complication. TDP1 could remove trapped topoisomerase I in a camptothecin-treated cell, only if the trapped complex had not yet been encountered by a moving DNA replication machine. If a collision had already occurred, TDP1 was powerless to repair the mess, and a different, more complicated and more imperfect repair process, such as homologous recombination, was needed to fix the problem. DNA repair by homologous recombination will be discussed later in Chapter ….

It turned out, however, that, in addition to cleaning off trapped topoisomerase complexes from the DNA, the TDP enzymes were able to clean off a variety of other anticancer drugs and toxin molecules that could bind and become trapped at the end of a DNA strand break (Pommier et al., 2014).

In sum, the TDP enzymes were shown to repair DNA that had suffered strand breaks to which extraneous molecular groups had become covalently attached. The TDP's were found capable of removing a surprisingly wide array of such groups. Their extensive repertoire was revealed first by their ability to remove topoisomerases that had become trapped at DNA breaks by anticancer drugs, such as doxorubicin and camptothecin. The scope of their DNA cleaning abilities was later shown to be much broader in terms of the kinds of strand-break-linked chemical entities they could cut away. Therapeutic applications were contemplated where TDP inhibitors might enhance the potency of drugs that trap DNA at strand breaks created by those drugs.

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